PATENT APPLICATION

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re the Application of

Paolo COZZI et al. Art. Unit: 1653

Serial No.: 09/623,506 Examiner: J.Russel

Filed: September 19, 2000 Atty.Docket.No.101615-007

For: ACRYLOYL DERIVATIVES ANALOGOUS TO DISTAMYCIN, PROCESS FOR PREPARING THEM AND THEIR USE AS ANTITUMOR AGENTS

DECLARATION UNDER 37 C.F.R. 1.132

Commissioner for Patents
P.O.Box 1450
Alexandria, VA 22313-1450

Date: **PROPOSED**

Sirs,

I, COZZI Paolo, hereby state and declare as follows:

I am inventor in the above-identified application, and an Italian citizen, residing at Via Zanella 48/5 – 20133 Milan, Italy.

In 1965, I graduated from the University of Milan and since 1995 have been employed by PHARMACIA & UPJOHN S.p.A:, an Italian corporation of Via Robert Koch, 1.2, 20152 Milan, Italy the Assignee of record in the present application. Since 1995 I have been engaged, in that Corporation, mainly in the field of anticancer DNA minor groove binders.

I am very familiar with the present invention, the above-identified application, the Office Action dated June 17, 2003, and the references cited therein.

Comparative experimental tests were performed under my supervision, to show IC50 values for a compound form the "Baraldi" reference and for compounds of the presently claimed invention. The "Baraldi" compound is structurally very close to the presently claimed compounds. The extent of structural similarity, in fact, is such that the compounds match almost entirely with the exception of the "B" group only, which therefore appears to be responsible for the increased pharmacological activity.

The experimental tests for demonstrating the IC50 values have been conducted from routine experiments carried out according to a well established assay procedure and, hence, are significant and highly reproducible.

The antitumor activity was evaluated in vitro by cytotoxicity studies carried out on murine L1210 leukemia cell. Cells were derived from in vivo tumors and established in cell culture.

Cells were used until the tenth passage. Cytotoxicity was determined by counting surviving cells after 4 hours treatment and 48 hours growth in drug-free medium.

The percentage of cell growth in the treated cultures was compared with that of controls. IC_{50} values (doses inhibiting 50% of the cellular growth in respect to controls) were calculated on dose-response curves.

As can be seen from the attached results of the tests, the compound 13 of Baraldi resulted in an IC50 of 9.9 ng/ml. In contrast, the compounds A and B of the present invention, which correspond with the compounds of Example 1 and 2, respectively, from page 21 and pages 24-25, respectively in the present specification achieved an IC50 of 7.8 and 5.6, respectively.

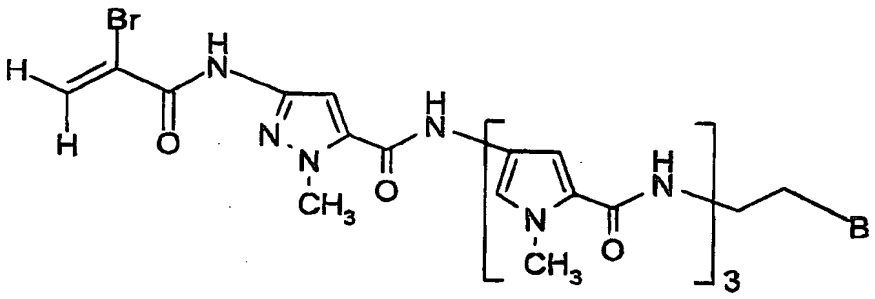
I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

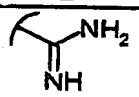
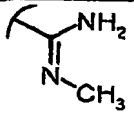
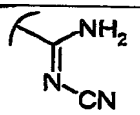
Signed this 30 October, 2003

Paolo Cozzi

Attachment: Results of Tests

Results of Tests



Compound	B	IC50 (ng/ml)
13 of Baraldi		9.9
Compound A		7.8
Compound B		5.6

Compounds A and B of the invention are reported in Example 1 (page 21 of WO 99/50265) and Example 2 (page 24,25 of the same), respectively.